CHAPTER 31

Good Manufacturing Practice for Sterile Products

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31.1 GENERAL

First, an important statement of fundamental principle: good manufacturing practice (GMP) is not merely a codified set of official guidelines or regulations (as in "the GMPs") promulgated and enforced by one or other of a number of regulatory bodies of varying degrees of expertise and competence. Its objective is not to satisfy, gratify or placate these bodies, rather its true and only meaningful objective lies in ensuring "... the safety, well-being and protection of the patient".[1] This is true for the manufacture of all categories of medicinal, drug, pharmaceutical or other health care products. It is most emphatically, most crucially true for the manufacture of *sterile* medicinal products. These products are among the most difficult to manufacture according to appropriate quality standards. They also have the potential, through faulty manufacture, to be the most hazardous to patients.

Sterile products are notably different from other, non-sterile, health care products. The difference lies quite simply and obviously in the fact that they are, or are at least *intended* to be, sterile. This predicates manufacturing, control and quality requirements additional to those that are relevant to non-sterile products. The same quality and GMP considerations that apply to non-sterile products apply equally well to sterile products. But the attainment and maintenance of the sterile state imposes extra quality assuring demands. That is, the special requirements (ethical and professional as well as regulatory) for sterile products manufacture are *additional to*, rather than separate from, those that apply to health care products in general. Worldwide, most official statements on GMP, with perhaps the surprising exception of the US drug products "cGMPs" (21 CFR Parts 210 and 211), have substantial separate sections on sterile products. The "Sterile Medicinal Products" Annex of the EC GMP Guide[2] is the largest single section in that publication.

31.1.1 What Does "Sterile" Mean?

It is surprising that so important a word, so central a concept, as "sterile" (or "sterility") has been so ill-defined and has met with such wooly ambiguity and equivocation in the various attempts that have been made to define it. (For a detailed analysis of this problem, see Sharp.[3])

A totally unambiguous and unequivocal definition appeared in the 1983 edition of the British Guide to GMP (the "Orange Guide")[1]

Sterility: The complete absence of living organisms. [Note: The state of sterility is an absolute-There are no degrees of sterility]

There have, however, been those who seem to find such a definition *too* uncompromising – and who prefer a concept of a state of "near ..." or "almost ..." sterility, where apparently the odd organism, here and there, is acceptable. The first official statement along these lines appeared in an amendment to the Nordic Pharmacopoeia in 1970:

"Sterile drugs must be prepared and sterilised under conditions which aim at such a result that in one million units there will be no more than one living micro-organism".

(Nordic Pharmacopoeia 1970)

This Nordic definition set a pattern for a number of other "one-in-a-million-is OK" definitions. The US Pharmacopoeia (USP) XXI, for example, declared

"(It is) generally accepted that ... injectable articles or ... devices purporting to be sterile ... (when autoclaved) attain a 10^{-6} microbial survivor probability, i.e. assurance of less than one chance in a million that viable organisms are present in the sterilised article or dosage form".
(USP XXI)

The British Pharmacopoeia (BP) in 1988 adopted a similar (but note, only an *approximately* similar) stance, in considering sterility to be

"... a theoretical level of not more than one living microorganism in 10^6 containers in the final product".

The potential problems (for patients) need to be considered before any unquestioning acceptance of such definitions. Consider, for example, a large volume parenteral (LVP) infusion. As real life events have tragically illustrated, in such products even normally non-pathogenic organisms can kill.[4] In many such products, one organism today can become many millions tomorrow. Over 100 million units of LVP solutions are administered annually throughout the world. Is it really acceptable that one in every million of those may contain organisms?– If it is, then we must also be prepared to accept 100+ unnecessarily dead patients per year.

However, more recent editions of USP and BP have evidenced a degree of backing away from the "less-than-one-in-a-million" position, possibly as a result of a dawning realisation of the potential practical consequences of adopting such a position. For example, the BP 2000 (Appendix XVIII) unequivocally states "Sterility is the absence of viable organisms" and adds

The sterility of a product cannot be guaranteed by testing; it has to be assured by the application of a suitably validated production process ... Failure to follow meticulously a validated process involves the risk of a non-sterile product or of a deteriorated product ... It is expected that the principles of good manufacturing practice (as described in, for example, the European Community Guide to GMP) will have been observed in the design of the process including, in particular, the use of:

- *qualified personnel with appropriate training*
- *adequate premises*
- *suitable production equipment, designed for easy cleaning and sterilization*
- *adequate precautions to minimize the Bioburden prior to sterilization*
- *validated procedures for all critical production steps*
- *environmental monitoring and in-process testing procedures.*

Wherever possible, a process in which the product is sterilized in its final container (terminal sterilization) is chosen. (Author's emphasis)

Later in this same Appendix (XVIII) the BP adds

"The achievement of sterility within any one item in a population submitted to a sterilisation process cannot be guaranteed, nor can it be demonstrated .... The SAL (Sterility Assurance Level) for a given process is expressed as the probability of a non-sterile item in that population. An SAL of 10^{-6}, for example, denotes a probability of not more than one viable microorganism in 1×10^6 sterilised items of the final product. The SAL of a process for a given product is established by appropriate validation studies".

Space will permit only the quotation of just two further examples of differing "definitions" of sterility:

"Out of a batch of one million units only one container may contain an organism (Statistically maximally 3 at a 95% confidence level)".[5]

And

"STERILITY is the absence of living organisms. The conditions of the sterility test are given in the European Pharmacopoeia". (EC GMP Guide)[2]

With the first clause of this EC definition, there can be no argument. Unfortunately, this statement is totally undermined by the immediately following implication that the so-called sterility test has relevance to the establishment of a state of sterility throughout a batch of product, which is, of course, nonsense, and dangerous nonsense at that.

Sufficient examples have been given (and there are others, see Sharp[3]) to demonstrate a range of variable (and indeed, often ambiguous) views on what is meant by the word "sterility". There can be no more crucial quality characteristic than the sterility of, say, a parenteral product; yet just precisely what "sterile" *means* seems to have not been entirely clear in the minds of a number of pundits. In many statements on the subject, there is an element of compromise. Sterility tends to be regarded by some, very wrongly, as a *conditional* rather than an *absolute* state.

What is the problem? Why this indecision and ambiguity over so fundamental an issue? The answer is a very simple, albeit philosophical, one. It is that there is a *fundamental flaw* at the heart of much thinking and writing about sterility, and *that flaw resides in a confusion between the nature of a concept or a state, and the probability of the existence of that state.*

The point was well made in an insufficiently noticed paper by Brown and Gilbert[6]:

"The concept of sterility is absolute. Whether or not a product is sterile is inevitably a matter of probability".

If this distinction were universally noted and adopted, the problem would cease to exist. The *only possible* definition of "sterility" is the uncompromised, unconditional and absolute one given in the UK Orange Guide, 1983. The question of the existence of such an absolute, negative state must, inevitably, be a matter of probability, not of absolute certainty, and there is nothing new or odd about that. This does not preclude our *aiming* to achieve this (or any other) absolute state. In the case of *sterility*, our concern should be about whether we have in fact achieved that state at an acceptable level of probability, and it is not unreasonable to suggest that what may be regarded as an acceptable level of probability could well be considered to vary according to circumstances.

Compare, for example, two different types of terminally heat-sterilised product:

(i) A small volume (say, 0.5 or 1.0 mL) injection of a *non*-growth supporting liquid, intended for intramuscular or sub-cutaneous injection – and –
(ii) An LVP, say 1 L of (growth supporting) dextrose/saline solution for intravenous infusion.

There can be no question that both should be sterile. However, one might well consider that the *level of assurance of the probability of attainment of that state* is more critical in the latter than the former.

31.1.2 Sterilisation – Fundamental Concepts

The process of sterilisation is often spoken of as if it were one single discrete type of operation. This is just not so. The various different methods of sterilisation are indeed very different one from the other: different in underlying theory and concept, in technology and in the advantages/disadvantages and potential hazards that each method represents. Each method needs to be operated and supervised by persons with a full understanding of, and experience of, that particular method and not merely of "sterilisation" in general. It is hardly an exaggeration to state, *e.g.* that the technological difference between the manufacture of sterile products using say (a) steam sterilisation and (b) filtration with aseptic processing is as great as, if not greater than, the difference between the manufacture of tablets and the manufacture of ointments.

There are two distinct, basic approaches for making a sterile product:

(i) Filling and sealing the product into its final container and then sterilising it (*terminal sterilisation*).
(ii) Sterilising a product at some earlier stage, before it is filled or packed, and then carrying out further processing and filling into sterile containers, using *aseptic* techniques and taking *aseptic* precautions.

It is generally considered that, if possible, it is best to use a terminal sterilisation process. This is simply because if the product is sterilised when securely sealed in its container it will remain sterile until the container is opened, broken or punctured in some way. When working with an unsealed sterile product, there is always a risk of re-contamination. Although that risk can be greatly reduced if proper care, with the application of modern technology, is taken, it can nevertheless be never entirely eliminated. That is why most experts and regulatory authorities agree that, where it is possible, products should be terminally sterilised. Sometimes this is not possible, for example when a product is not able to withstand a terminal heat-sterilisation process.

In addition to the obvious objective of making sterile products, which are *in fact* sterile, the products are also required, in many cases, to be free of particles and of pyrogens (or "bacterial endotoxins"). The attributes of freedom from organisms, non-viable particles and pyrogenic substances may be said to be interconnected. (Air-borne microorganisms are frequently associated with non-viable particles and pyrogens usually originate from the cell walls of Gram-negative bacteria.)

Another fundamental point, which is relevant to all types of sterilisation, is that *sole reliance cannot be placed on the sterilisation process alone, in isolation, to achieve sterility*. Much depends on

- The microbial condition of the materials, or articles, as they are presented to the sterilisation process
- On how they are prepared and handled *before* the actual sterilisation, by whom and under what conditions
- On the pre-established validity of the sterilisation process itself

- On the careful control of that process during the sterilisation and
- On what happens after the sterilisation process to confirm its efficacy and to prevent product re-contamination

The fragile fallibility of the so-called "Sterility Test" as a weak support in the assurance of sterility is so universally acknowledged that it requires no further emphasis. It is the very fact of the lack of any meaningful end-product test to demonstrate the sterility of a batch, which necessitates more disciplined approaches and higher orders of care and attention. Crucial to success are

- The *people* involved and their *training*
- The *premises* used and the *environmental standards* therein
- The *equipment* and its *commissioning*/*cleaning*/*sterilisation*
- The quality of the *materials* used (including *water*)
- *Validation* of the sterilisation process
- *In-process control* of the process and *in-process control* of the manufacturing environment.

31.2 PREMISES FOR STERILE PRODUCTS MANUFACTURE

31.2.1 Clean Rooms

The concept and design of clean rooms was first developed (in the early 1960s) for the microelectronic and aerospace industries. In these industries, it is important to protect microelectronic components against even the finest particles. Viable contaminants, as such, are of no special significance to microcircuits, only in so far as bacteria, *etc*. are themselves particles.

A clean room, as originally conceived, may be defined as follows:

A clean room is an enclosed space with quantitatively specified control of:

- *Particles*
- *Temperature*
- *Pressure*
- *Humidity*

constructed with non-porous surfaces which are easy to clean and maintain, with controlled access via air-locks, and operated in accordance with procedures designed to keep contamination below a defined low level.

Because of this origin in industries where product *sterility* is not the aim, classifications of the various classes of clean room are usually based upon the number and size of the particles (purely as *particles*) permitted per unit volume of the air in the room. Many published clean room standards also have specifications for humidity, temperature, lighting and air pressure. It was only later that the pharmaceutical and related industries adopted the concept, and then added to it certain permissible levels of microbial (or viable) contamination. Even so, a lot of what is said and written on this subject still sounds or reads as if it were based on a premise that it is the inanimate particles that are crucially important. In fact (all other things being equal), the presence or absence of *viable* contamination in, say, a parenteral product is a quality and patient safety issue which is even more critical than the presence or absence of non-viable contamination. It has to be said, however, that although there has been a lack of convincing demonstration of a direct linear relationship between the numbers of non-viable and the numbers of viable particles in a given volume of air, it is entirely reasonable to suppose that where there are low levels of non-viable particles there will

concomitantly be low levels of microorganisms. Air-borne microorganisms are most characteristically to be found associated with particles or droplets.

The unit of measure used to define clean rooms is the micrometre (0.001 mm), very commonly termed a ''micron''. The first official published standard for clean rooms was the US ''Federal Standard 209: Clean Room and Work Station Requirements, Controlled Environment''. This standard has gone through a number of revisions over the years since the 1960s – 209B, 209C, 209D and 209E, but the basic idea behind the US classification has remained the same. It is based on permitted numbers, per *cubic foot* of air, of particles of a size 0.5 μm and larger.

There are three classes of US Federal Standard 209 Clean Room, which are particularly relevant to sterile products manufacture: class 100, class 10,000 and class 100,000 (see Table 1).

This US standard defines a number of other clean room parameters and conditions, such as temperature, humidity, air pressure, operator clothing and behaviour, and the instruments and devices to be used to measure and count particles in the air, but to date, it has made no reference to permitted levels of microorganisms.

Following the lead given by the US Federal Standard 209, a number of national and other bodies have produced standards for clean rooms, in essence very like the US standard, but with changes in the nomenclature used for the various classes, or *Grades*. For example, in 1976, British Standard No. 5295 ''Environmental Cleanliness in Enclosed Spaces'' was published. This defined four main classes of clean room. In sterile products manufacturing areas, normally only the first three of them are of interest or concern, *class* 1, *class* 2 and *class* 3.

While at first sight these may look different, they are (respectively) closely similar to the Federal Standards – *class* 100, *class* 10,000 and *class* 100,000, with, in British Standard 5295 the permitted number of particles in each class expressed in terms of a cubic metre of air, not per cubic foot. The BS 5295 (1976) figures for the three classes are shown in Table 2.

In 1989, a revised version of BS 5295 was issued with the various classes designated by letters rather than numbers, and the current edition of the Federal Standard (209E) has ''gone metric'', and expresses the numbers of particles permitted, in each class, in terms of a cubic metre, while still retaining the old per cubic foot basis. Thus, ''Class 100'' is now also expressed as ''Class M 3.5'' (note: ''M'' here stands for ''metric'' and not ''microbial'').

Table 3 shows how the standards so far discussed, plus some others, relate more or less to one and other.

Table 1 *US Federal Standard 209E – maximum permitted number of particles per cubic foot of air in room*

Class	*Particle size*	
	≥ 0.5 μm	*≥ 5 μm*
Class 100	100	0
Class 10,000	10,000	65
Class 100,000	100,000	700

Table 2 *BS5295 clean room standard – maximum permitted number of particles per cubic metre of air in room*

Class	*Particle size*			
	≥ 0.5 μm	*≥ 1.0 μm*	*≥ 5.0 μm*	*≥ 10 μm*
Class 1	3000	N/A	0	0
Class 2	300,000	N/A	2000	3
Class 3	N/A	1,000,000	20,000	4000

Table 3 *Environmental standards (approximate comparisons)*

Federal Standard 209	Class:	100	10,000	100,000
Federal Standard 209E	Class:	M 3.5	M.5.5	M.6.5
British Standard 5295 (1976)	Class:	1	2	3
Pharmaceutical Inspection Convention (1981)	Grade:	A/B	C	D
UK ''Orange Guide''	Grade:	1(A/B)	2	3
EC Guide (1989/1992)	Grade:	A/B	C	D
British Standard 5295 (1989)	Class:	E/F	J	K

31.3 CLEAN ROOM STANDARDS AND GMP GUIDELINES

The US CGMPs for finished pharmaceuticals[7] make no mention of clean rooms, in the specific sense, and offer no quantitative standards, for neither viable nor non-viable particles.

Sub-part C (Buildings and Facilities) includes, under Section 211.42 (Design and construction features), the following:

> *(c) Operations shall be performed within specifically defined areas of adequate size. There shall be separate or defined areas or such other control systems for the firm's operations as are necessary to prevent contamination or mix-ups during the course of the following procedures:*
>
> *(10) Aseptic processing, which includes as appropriate:*
>
> *(i) Floors, walls, and ceilings of smooth, hard surfaces that are easily cleanable;*
>
> *(ii) Temperature and humidity controls;*
>
> *(iii) An air supply filtered through high-efficiency particulate air filters under positive pressure, regardless of whether flow is laminar or non-laminar;*
>
> *(iv) A system for monitoring environmental conditions;*
>
> *(v) A system for cleaning and disinfecting the room and equipment to produce aseptic conditions;*
>
> *(vi) A system for maintaining any equipment used to control the aseptic conditions.*

Sub-part C (Buildings and Facilities) adds, under Section 211.46 (Ventilation, air filtration, air heating and cooling), the following:

> *(a) Adequate ventilation shall be provided.*
>
> *(b) Equipment for adequate control over air pressure, microorganisms, dust, humidity, and temperature shall be used when appropriate ....*
>
> *(c) Air filtration systems, including pre-filters and particulate matter air filters, shall be used when appropriate on air supplies to production areas.*

The FDA ''Guideline on Sterile Drug Products Produced by Aseptic Processing'' (FDA, 1987)[8] is somewhat more specific. It does not refer to ''Clean Rooms'' as such, but it does draw a distinction between a ''Critical Area, ... in which the sterilised dosage form, containers and closures are exposed to the environment'', and a ''Controlled Area, where unsterilised product, in-process materials, and container/closures are prepared''.

According to this FDA guideline, air in a critical area, ''in the immediate proximity of exposed sterilised containers/closures and filling/closing operations is of acceptable quality when it has a per-cubic-foot particle count of no more than 100 in a size range of 0.5 μm and larger (class 100) when measured not more than one foot away from the work site, and upstream of the air flow ...''. In a critical area the air ''should also be of a high microbial quality. A incidence of no more than one colony forming unit per 10 cubic feet is considered as attainable and desirable''.

This FDA guideline considers that, in a controlled area, the air ''is generally of acceptable particulate quality if it has a per-cubic-foot particle count of not more than 100,000 in a size range

of 0.5 μm and larger (class 100,000) when measured in the vicinity of the exposed articles during periods of activity. With regard to microbial quality, an incidence of no more than 25 colony-forming units per 10 cubic feet is acceptable''. (At the time of writing it is understood that a revised version of this FDA guideline is in an advanced stage of preparation.)

The 3rd edition of the UK GMP Guide (1983) set out, probably for the first time in any official GMP guideline, its own ''Basic Environmental Standards for the Manufacture of Sterile Products''. These were based on the British Standard 5295 (1976), but with the addition of a series of maximum permitted levels of viable organisms per cubic metre of air.

These standards were summarised in a table, which in addition to giving levels for particles generally, which are similar to those of BS 5295 (1976), also gave figures for

- Air-filter efficiency
- Air changes (per hour) in the room
- Viable organisms per cubic metre

There is also a cross reference to other classifications, including BS 5295 and US Federal 209 and a note that air pressures should ''always be highest in the area of greatest risk'', and that ''air pressure differentials between rooms of successively higher to lower risk should be at least 1.5 mm (0.06 inch) water gauge'' (*i.e.* approximately equivalent to a 15 Pa air pressure differential). This exemplified the concept of the air pressure ''cascade''.

The European Community GMP Guidelines (1989/92) refer to *Grades* A, B, C and D, and defined these in tabular ''Air classification system for the manufacture of sterile products'', as shown in Table 4.

In 1996, a revised version of Annex 1 (''Manufacture of Sterile Medicinal Products'') to the EC GMP Guide was issued, which some have felt serves rather to cloud the issue than to clarify it.

This revised EC Annex offers a new tabular classification for environmental particle levels (see Table 5). It is noteworthy that this table differs from its British and EC antecedents in, as it were, ''divorcing'' inanimate particulate requirements from microbial limits. Hitherto, for example, ''Grade B'' meant, in one cubic metre of air, no more than 3500 particles at the 0.5 μm level, none at 5 μm *and* no more than 5 microorganisms, and so on. Now in this 1996 classification, a given Grade (A, B, C or D) refers *only* to the permitted inanimate particle levels. Furthermore, two sets of figures (different except, obviously, for Grade A) are given. One for the ''at rest'' state and one for the ''in operation'' state (see Table 5).

Textual comment, printed in this annex in relation to this table, reads as follows:

> *''For the manufacture of sterile medicinal products normally 4 grades can be distinguished.*
> *Grade A: The local zone for high risk operations, e.g. filling zone, stopper bowls, open ampoules and vials, making aseptic connections. Normally such conditions are provided by a*

Table 4 *EC GMP Guide (1992) air quality standards*

	Maximum permitted number of particles per cubic metre		*Maximum permitted number of viable microorganisms per cubic metre*
Grade	≥ 0.5 μm		
A (LAF workstation)	3500	None	<1[a]
B	3500	None	5[a]
C	350,000	2000	100
D	3,500,000	20,000	500

[a] Reliable when only a large number of air samples are taken.

Table 5 *Airborne particulate classification' EC GMP Guide, Annex 1 (revised 1996)*

Grade	*Maximum permitted number of particles per cubic metre*			
	At rest (b)		*In operation*	
	≥ 0.5 μm	*≥ 5 μm*	*≥ 0.5 μm*	*≥ 5 μm*
A	3,500	0	3,500	0
B (a)	3,500	0	350,000	2,000
C (a)	350,000	2,000	3,500,000	20,000
D (a)	3,50,0000	20,000	Not defined (c)	Not defined(c)

laminar airflow workstation. Laminar airflow systems should provide a homogenous air speed of 0.45 m/s +/− 20% (guidance value) at the working position.

Grade B: In the case of aseptic preparation and filling, the background environment for Grade A zone.

Grade C and D: Clean areas for carrying out less critical stages in the manufacture of sterile products.

Notes appended to the table, and keyed to "(a)", "(b)", "(c)" and "(d)" as they appear in that table, read as follows:

(a) In order to reach the B, C and D air grades, the number of air changes should be related to the size of the room and the equipment and personnel present in the room. The air system should be provided with appropriate filters such as HEPA for grades A, B and C.

(b) The guidance given for the maximum permitted number of particles in the 'at rest' condition corresponds approximately to the US Federal Standard 209E and the ISO classifications as follows: grades A and B correspond with class 100, M 3.5, ISO 5; grade C with class 10,000, M5.5, ISO 7 and grade D with class 100,000, M6.5, ISO 8.

(c) The requirement and limit for this area will depend on the nature of the operations carried out.

No mention is made of differential room air pressures and required air changes are not quantified.

Further, three tables are also provided in this EC Annex. Two give "examples of operations to be carried out in the various grades", in relation to terminally sterilised products and aseptic preparation, respectively, and the third provides "Recommended limits for microbial monitoring" in the different grades of room.

The two given "examples of operations to be carried out in the various grades" are shown in Tables 6 and 7, and the third ("Recommended limits for microbiological monitoring ...") in Table 8.

There is no need to expatiate on the ambiguities and imprecisions represented by these tables. The reader will surely have noted them (for a cogent analysis of this revised EC Annex, see Walker[9]).

The textual matter relating to these tables, as reproduced in Tables 6 and 7 *may* provide some clarification. It reads

"The particulate conditions given in the table for the "at rest" state should be achieved in the unmanned state after a short "clean-up" period of 15–20 minutes (guidance value) after completion of operations. The particulate conditions for grade A in operation given in the table should be maintained in the zone immediately surrounding the product whenever the product or open container is exposed to the environment. It is accepted that it may not always be possible to

Table 6 *After EC Guide Annex 1, revised 1996*

Grade	*Examples of operations for terminally sterilised products*
A	Filling of products when unusually at risk
C	Preparation of solutions when unusually at risk
D	Preparation of solutions and components for subsequent filling

Table 7 *After EC Guide, Annex 1, revised 1996*

Grade	*Examples of operations for aseptic preparations*
A	Aseptic preparation and filling
C	Preparation of solutions to be filtered
D	Handling of components after washing

Table 8 *Recommended limits for monitoring of clean areas in operation (EC GMP Guide, Annex 1, revised 1996)*

Grade	*Air sample (cfu per m^3)*	*Settle plates (diameter 90 mm) cfu per 4 h*	*Contact plates (diameter 55 mm) cfu per plate*	*Glove print, 5 fingers cfu per glove*
A	>1	>1	>1	>1
B	10	5	5	5
C	100	50	25	–
D	200	100	50	–

demonstrate conformity with particulate standards at the point of fill when filling is in progress, due to the generation of particles or droplets from the product itself''.

Further comment on the application of the various grades appears in the sub-sections on ''Terminally sterilised products'' and on ''Aseptic preparation'' and thus

''Terminally sterilised products: Preparation of components and most products should be done in at least a grade D environment in order to give low risk of microbial and particulate contamination, suitable for filtration and sterilisation. Where there is unusual risk to the product because of microbial contamination, for example, because the product actively supports microbial growth or must be held for long periods before sterilisation or is necessarily processed not mainly in closed vessels, preparation should be done in a grade C environment.

Filling of products for terminal sterilisation should be done in at least a grade C environment. Where the product is at unusual risk of contamination from the environment, for example because the filling operation is slow or the containers are wide-necked or are necessarily exposed for more than a few seconds before sealing, the filling should be done in a grade A zone with at least a grade C background. Preparation and filling of ointments, creams, suspensions and emulsions should generally be done in a grade C environment before terminal sterilisation.

Aseptic preparation: Components after washing should be handled in at least a grade D environment. Handling of sterile starting materials and components, unless subjected to sterilisation or filtration through a microorganism-retaining filter later in the process, should be done in a grade A environment with grade B background.

Preparations of solutions which are to be sterile filtered during the process should be done in a grade C environment; if not filtered the preparation of materials and products should be done in a grade A environment with a grade B background.

Handling and filling of aseptically prepared products should be done in a grade A environment with a grade B background.

Transfer of partially closed containers, as used in freeze drying should, prior to the completion of stoppering, be done either in a grade A environment, or in sealed transfer trays in a grade B environment.

Preparation and filling of sterile ointments, creams, suspensions and emulsions should be done in a grade A environment, with a grade B background, when the product is exposed and is not subsequently filtered''.

There remains to be considered the revised EC Guide Annex view of what we have termed the ''divorced'' environmental microbial standards. Paragraph 5 of the revised Annex reads, *inter alia*

''5. In order to control the microbiological cleanliness of the various grades in operation, the areas should be monitored. Recommended limits for microbiological monitoring of clean areas in operation ... are ...''. (see Table 8)

31.3.1 The Sterile Products Manufacturing Area or "Suite"

It cannot be repeated too often that great care must be taken to protect the product from contamination *throughout the entire manufacturing process*, and that it is not sufficient merely to rely on a final filtration and/or sterilisation to ''clean things up''. It certainly *cannot* be hoped that if it fails to do so, then end-product testing, particularly by the notably fallible sterility test, will detect any problems. Thus, sterile products are manufactured in specially designed sterile manufacturing areas, or ''Suites'' of one or more clean rooms.

Within a sterile products suite, the various different manufacturing operations must be performed in a clean room (or rooms) appropriate to a particular operation. An air pressure ''cascade'' should be in operation, such that the air pressure is highest in the zone of potentially greatest risk (*e.g.* where exposed product is aseptically handled) and is progressively lower in areas of lesser risk. The entire suite should be at a higher air pressure than the general factory environment and/or ''the outside world''.

31.3.2 Air Supply

The air supplied to various rooms must be of quantitatively specified quality. Air from the outside world, or from the rest of the factory, is neither suitable nor acceptable. Hence, no windows (or certainly no *openable* windows) to the outside are permissible. It follows that the air to the various rooms must be a forced supply, delivered via ventilation trunking, through filters designed and tested to ensure that air that has passed through them is of the required quality (that is, contains no more than the specified number of particles, and organisms, per cubic foot or cubic metre).

While it is common for coarser pre-filters to be used, in order to reduce the clogging of the final filters, it is important that the final air filters should be fitted at, or as close as possible to, the point of entry of the air into a room. This is usually at various places in the ceiling. It is also usual to design clean rooms so that it is possible to change these filters, as and when necessary, without having to do so from within the room itself (that is, for example, by making the change from above a ''false'' or suspended ceiling). This is to avoid contaminating the room with particles from the ''dirty'' side of the filter and to avoid no more personnel than absolutely necessary from having to enter the room.

It is generally considered that there should be a pressure differential between rooms of successively higher to lower risk of at least 15 Pa (approximately 1.5 mm water gauge = 0.06 in. water gauge), although some authorities (see, *e.g.* BS 5295) consider that a pressure differential of 10 Pa between a one classified area and another adjacent one of lower classification is acceptable, provided that the differential between the classified areas and adjacent unclassified areas is at least 15 Pa. Thus, the air pressure in the aseptic fill room should be higher than in solution preparation, and the pressure in both should be higher than in the changing rooms, which should in turn be at least 15 Pa higher than in the general unclassified area.

Pressure sensing devices (water gauges, manometers) must be installed to show the pressure differentials between rooms, and there should be audible or visible warning systems which sound, or display, alarm signals if the air supply fails and the required pressure drops.

Designing and installing air-supply systems is a highly specialised business, as is the balancing of air pressures so as to achieve the correct differentials and flows. Most usually clean rooms are constructed and installed, under contract, by specialists in this field. Careful selection of an appropriate specialist is crucial to success. Just as important is a clear, precise definition of just exactly what is wanted, and close and careful monitoring of the project, as the contract proceeds. When the installation has been completed and commissioned, it should be ''handed-over'', complete with a certificate of conformity to specification. This should contain certification of at least.

(i) Air-filter integrity – all air-inlet filters tested to confirm filter and seal integrity, and conformity to the specified standard.
(ii) Air velocity – measured by anemometer to determine air velocity (m s^{-1}) at the internal filter face of each air inlet.
(iii) Air change rate – calculated for each clean room from the air velocity and the internal volume of the room.
(iv) Air particle counts – as measured in each clean room, in terms of the number of particles (of specified size) per cubic metre, or cubic foot, at the positions and heights specified in the standard against which the clean rooms were constructed and commissioned.

With, in addition, reports of checks on airflow patterns, room-pressure differentials, lighting levels, heating and humidity.

Once the system has been installed and the suite is operational, it is necessary to continue to check and monitor air-filter (and seal) integrity and efficiency and that the air pressures and flows remain as required and as specified. It is also necessary to check airflow rates at filter faces and room air change rates (which should be at least 20 air changes per hour).

There will, of course, be people working in these clean rooms. There therefore needs to be heating or cooling of all this high-quality air to ensure the right level of comfort, particularly for operators clothed in the special clean room garments, which can make them uncomfortably hot. It is important that operators do not get too warm, since the more they sweat, the more particles and organisms they will shed.

The quality of the air, and surfaces, within the rooms must also be regularly monitored.

Total particles per unit volume of air may be determined by drawing a sample of air (of known volume) through a gridded filter membrane (capable of retaining particles of at least the size under investigation, *e.g.* of at least 0.5 μm), and then examining the membrane under a microscope for the size and numbers of particles. This is the reference method for demonstrating compliance with BS 5295. However, more often used are the various commercial brands of optical or laser particle counters.

Microbial levels (air-borne or on surfaces) in a clean room may be determined by use of:

- Settle plates
- Air samplers

- Surface sampling
- "Finger dabs".

Settle plates are petri dishes containing sterile nutrient agar. The plates are most usually 90 mm in diameter (surface area approximately 0.006 m^2), although plates of 140 mm diameter (approximately 0.015 m^2 surface area) have been used. It is thus necessary when reporting settle-plate results (and when establishing standards for settle-plate counts) to state both the size of the plate (s) and the time of exposure. For valid comparisons to be made between results obtained using different plate sizes, the results should be expressed as cfus 100 cm^{-2} h^{-1}.

It has been argued that settle plates do not give a measure of the concentration of microorganisms in the air of a room. This is true, but it may equally be argued that, in providing a direct measure of the organisms which are depositing from the air and on to surfaces (or into containers), they do provide an indication of what the sterile product manufacturer really requires to know – the likely microbial contamination entering into, or onto, products (see Whyte[10]).

Air samplers are commercially available in a number of different types: cascade samplers, slit-to-agar samplers, single-sieve-to-agar samplers, centrifugal samplers, filtration samplers and liquid impingement samplers. In all these air sampling methods, knowledge of the sampling rate, the time period over which the sample was taken, and of the number of cfus counted after incubation will enable the determination of the number of viable organisms present in unit volume of air in the room.

31.3.3 Surface Sampling

Surfaces of walls, floors, work and equipment surfaces can be sampled using moistened sterile cotton swabs, which are then "streaked-out" on an agar plate, which is then incubated. Alternatively, and more conveniently except for less-accessible surfaces, contact (or "Rodac") plates can be pressed lightly onto flat surfaces and incubated. Following the application of a swab, or a contact plate, the relevant surface area should be wiped with a disinfectant wipe.

31.3.4 Finger Dabs

Although "finger dabs" do not directly measure the microbial contamination of the air in a clean room, they do give an indication of the contamination picked up by operators from surfaces in the room.

After, or as, they leave the room, operators touch the tips of all digits of both gloved hands onto an agar plate, which is then incubated. (It should go without saying that the gloves should be discarded and fresh ones put on before an operator continues to work.)

A similar technique can be employed as a training exercise, outside the sterile area, by applying operators' ungloved fingers (and indeed noses, ears or whatever) to an agar plate to provide them with a graphic illustration of the organisms present on the human body surface.

31.3.5 Frequency of Monitoring/Checking of Clean Room Parameters

31.3.5.1 Physical.

(i) Room pressure differentials – There should be a continuous automatic manometric measurement linked to unmistakable visual and/or audible warning signals that are triggered whenever pressure drops below the specified level. The manometer gauges should also be visually checked hourly, and the reading recorded at least once per day (or per shift). It is essential that the manometers are regularly calibrated.

(ii) Air velocity and room air change rates – performed and recorded every 6 months.

(iii) Air particle counts – performed daily (or batchwise) in the more critical areas, and weekly in the less.
(iv) Air filter integrity and efficiency test – carried out once or twice a year, unless results of in-process physical and microbial monitoring indicate a more urgent need.

Airflow directions and patterns should also be occasionally checked, as convenient.

31.3.5.2 Microbial. While there is something like general agreement on the frequency of monitoring of physical clean room parameters (for example, frequency rates similar to those set out above are to be found in BS 5295), no such official or general agreement exists with regard to frequency of microbial monitoring. In these circumstances, the following seems to be a reasonable schedule for the different levels of clean room:

- At contained workstations (A) – daily or batchwise
- In clean rooms grade B/Cl.100 – daily
- In clean rooms grade C/Cl.10 000 – weekly
- In clean rooms grade D/Cl. 100 000 – weekly, without all aspects of microbial monitoring necessarily being carried out on each occasion.

(For more detailed information the reader is referred to the Technical Monograph No. 2 of the Parenteral Society, 1989.[11])

31.4 THE STERILE MANUFACTURING AREA – CONSTRUCTION AND FINISHES

The surfaces of all floors, walls and ceilings should be hard, smooth, impervious and unbroken (*i.e.* no cracks, holes or other damage). There are three good reasons for this:

(i) To prevent the shedding of particles from damaged or poorly finished brick, building block, plaster, *etc.*
(ii) To prevent the accumulation of dust, dirt and microorganisms on, or in, rough or broken surfaces.
(iii) To permit easy and repeated cleaning and disinfection.

Various materials have been used for floors, including welded sheet vinyl, terrazzo and various "poured" resin floors. A variety of basic structural materials are used for walls – bricks, blocks, plastic-coated metal panels and glass-reinforced plastics. All are acceptable, provided that the final finish provides a *smooth, impervious, unbroken* surface. Thus if a wall is constructed of brick or structural block, it must be smooth plastered and then coated with a hard-setting finish (polyurethane, epoxy, *etc.*), sprayed or painted on.

Welded sheet vinyl is also used as a wall finish, often as a continuation of the same material when it has also been used as a floor surface.

Where windows are installed, they should not be openable. They should be flush fitted on the controlled (or classified) area side. Where windows are fitted in a dividing wall between two classified areas or rooms, they should be double glazed so as to present a flush, ledge-less fit on both sides. If communication is necessary between adjacent clean rooms, this should be via "speech panels" (polymeric membranes that transmit sound while maintaining an airtight seal). They can be used back-to-back in double glazed windows. When installed in the more critical clean rooms, the usual protective grilles should be removed, as they are difficult to clean. Telephone and intercom installations should generally be avoided, certainly in aseptic processing rooms. If they are deemed

essential, in for example a solution preparation room, they should be purpose designed, flush mounted and with easily wipeable touch-sensitive controls.

Ceilings in sterile areas are often "false" or suspended to allow for the installation of air-supply ducting, and other services, above. It is important that any suspended ceiling is effectively sealed from the room below to prevent any possible contamination from the space between the false and the "real" ceiling.

Where floors meet walls and walls meet ceilings, the joins should be covered so as to avoid sharp corners that are difficult to clean, and it can harbour dust, dirt and microorganisms. It is also important that any such coving should be flush to both floor and wall (or wall and ceiling).

31.5 PERSONNEL

Even more than in relation to other types of manufacture, the *people* involved are the most important single factor. The person, or persons, who manage sterile products departments should have a full understanding of, and experience in, the special techniques, technologies and disciplines required – and of the underlying physical, chemical, microbiological and clinical reasons. They should be able to impart their knowledge and understanding to their staff, who should also be selected with care. Workers in sterile products areas should be mature (and that does not necessarily mean old) intelligent people who can fully understand not just what they have to do, but also the reasons for doing it. They must have innately high standards of personal hygiene and be readily able to conform to the special disciplines involved. They should also be free from any disease or condition that could represent an abnormal microbiological hazard to the clean room environment, and hence to the product. These conditions include, in addition to chronic gastro-intestinal and respiratory tract diseases, short-term conditions such as colds, acute diarrhoea, skin rashes, boils, open superficial injuries and peeling sunburn. Operators should be required to report any such conditions, and supervisory staff should be on the look-out for them. There should be periodic health checks.

In addition to those who have chronic skin, respiratory or gut diseases, persons who have allergies to the synthetic fabrics used in clean room clothing, are abnormally high shedders of skin flakes or dandruff, have nervous conditions resulting in excessive itching, scratching, *etc*. or suffer from any degree of claustrophobia, are really not fitted to work in clean rooms.

No person who reports that they have a condition that would preclude their working in a clean area should suffer any penalty for doing so. The thought of loss of earnings might well persuade even the most virtuous worker to keep quiet about an adverse health condition.

A certain calm resoluteness of character is also most desirable. To be alone, or perhaps be just one of two or three in a clean room, in a full sterile suit with gloves, hood, mask and possibly goggles can prove a lonely, depressing, demotivating experience for some temperaments. Conversely, while a cheery, fun-loving, whistle-as-you-work attitude may well be salutary in some areas of human activity, it is entirely inappropriate in a clean room.

To minimise the contamination inevitably caused by the presence of people, the numbers entering and working in clean rooms should be kept to the minimum necessary for effective working. All activities, such as in-process testing and control, visual inspection and the like, which do not need to be conducted in clean room should be performed outside it.

All personnel, and that includes cleaning staff and maintenance engineers, required to work in, or otherwise enter a clean area should be trained in the techniques and disciplines relevant to the safe and effective manufacture of sterile products. This training, which should not be a "one-off" exercise but should be regularly reinforced with refresher training, should include the coverage of personal hygiene, the essential elements of microbiology and the purpose and correct wearing of protective clothing. Operators should be taught to "know the enemy", and practical

demonstrations of growing cultures, finger dabs and the like will help to get the message home. Training should also include a strong motivational element, stressing responsibilities to patients' health and life, which are quite literally "in your hands".

Any outside persons such as building or maintenance contractors, who have not received the training and who need to enter clean areas, should only do so under close supervision and when wearing protective clothing appropriate to the area.

31.5.1 Personnel – Changing and Clothing

Personnel should only enter a clean area *via* changing rooms, where washing and changing should proceed in strict accordance with a written procedure. The operators should have been trained to follow this procedure, and a copy of it clearly displayed on the changing room wall. The procedure should be designed to minimise contamination of the protective clothing through, for example, contact with the floor on the "dirtier side" or with operators' shoes. Outdoor clothing should not be taken into clean room changing rooms. The assumption should be that outdoor garments have already been removed elsewhere, and that personnel are already clad in the standard "general factory" protective clothing. Wristwatches and jewelry should be removed as part of the changing process. Plain, simple wedding rings are generally considered to be an exception that is reasonable, sympathetic as well as expedient as many people find it impossible (physically or emotionally) to remove their wedding rings. However, the FDA are said not to agree on this point. Cosmetics, other than perhaps simple particle-free non-shedding creams, should not be worn.

The protective garments, which should include head and footwear, should be made from textiles specially manufactured so as to shed virtually no fibres or particles, and to retain any particles shed by a human body within. They should be comfortable to wear and loose-fitting to reduce abrasion. Fabric edges should be sealed and seams all enveloping. Unnecessary tucks and belts should be avoided, and there should be no external pockets. The garments should be worn only in the clean areas. A fresh set of clean (and if necessary sterilised) protective garments should be provided each time a person enters or re-enters a clean room. This should rigorously be enforced where aseptic processing is in operation. In other, less critical, clean rooms, it may be possible to relax this requirement and provide fresh garments once per day, if this can be justified on the basis of monitoring results and other control measures. Even so, fresh headwear, footwear and gloves should be provided for each working session.

Protective clothing, following use, should be washed or cleaned (and as necessary sterilised), and thereafter handled in such a way so as to prevent it from gathering contaminants and to minimise attrition of the fabric. It needs to be recognised that repeated wearing and laundering/cleaning (and sterilisation) can cumulatively damage the fabric so that it becomes no longer suitable for use. This is clearly something that needs to be monitored and controlled.

In grade C and D clean rooms one- or two-piece trouser suits should be worn, close fitting at the neck, wrists and ankles and with high necks. Hair, including any facial hair (beard or moustache), should be covered. Trouser bottoms should be tucked into overshoes or boots and sleeves into gloves.

In grade B clean rooms and/or when working at contained workstations, sterilised non-shedding cover-all trouser suits (preferably one piece) should be worn. Headwear should be of the helmet or cowl type and should totally enclose the hair and any beard/moustache. It should be completely tucked into the neck of the suit. Footwear should be of the boot or "boot-ee" type, totally enclosing the feet. Trouser bottoms should be completely tucked into the footwear. Powder-free rubber or plastic gloves should be worn with the garment sleeves neatly and completely tucked inside the gloves. Gloves should be regularly disinfected (*e.g.* with a sterile alcoholic spray or foam) during extended operations. Disposable face masks, covering both the nose and the mouth should be

worn. They should be discarded at least each time the wearers leave the clean room and whenever they become soggy. In the latter circumstances, it is of course necessary to leave the clean room to change the mask. Operators should be trained not to touch masks, or any other part of their face, with their hands when in a clean room.

Some authorities hold that, when working in an aseptic processing area, operators should wear close-fitting goggles. Indeed, the US FDA has been known to insist upon it. There are, however, those who would argue that any benefit, in terms of reduction in contamination hazard, is outweighed by the risks introduced by the additional operator discomfort and the misting of the goggle lenses.

When working at contained LAF workstations, operators should always work down-stream of the filter face and of any product, material or equipment that is being processed or manipulated at the work station. In other words, work should be conducted so that any operator-derived contamination is swept in a direction away from the work in hand. Hands or arms should not be interposed between the filter face and the product, as this would cause the air stream to sweep contamination from the operator onto the work – the very reverse of what is required.

There should be written instructions to operators on entering and working in clean rooms. These instructions should be used as a basis for training. They should also be prominently displayed in changing rooms. The following is intended to give an idea of the sort of thing that is necessary. It will also provide a summary of basic requirements for personnel in clean rooms.

31.6 INSTRUCTIONS TO OPERATORS ON ENTERING AND WORKING IN CLEAN ROOMS

(i) Keep body, hair, face, hands and fingernails clean.
(ii) Report any illnesses, cuts, grazes or respiratory, gut or skin problems.
(iii) Follow the written changing and wash-up procedure *exactly*.
(iv) Check that your protective clothing is worn properly.
(v) Do not wear cosmetics, jewelry or wristwatches.
(vi) Leave all personal items (wallets, coins, keys, watches, tissues, combs, *etc*.) in the changing room.
(vii) Do not take papers, documents or paper materials into clean rooms, unless these have been specifically approved.
(viii) *No* eating, chewing or drinking.
(ix) Always move gently and steadily.
(x) Do *not* move vigorously. *No* playing about, singing or whistling.
(xi) Avoid talking unless absolutely necessary.
(xii) Avoid coughing or sneezing. If these are unavoidable, leave the clean room.
(xiii) Do not touch other operators.
(xiv) Avoid scratching, touching nose and mouth and rubbing hands.
(xv) Where gloves are worn, regularly disinfect them as instructed.
(xvi) Always check for worn or damaged garments and torn gloves and change them as necessary.
(xvii) Keep garments fully fastened up. Do not unfasten or loosen them.
(xviii) Unless there is a special hazard involved, do not pick up dropped items from the floor.
(xix) When working at a laminar flow workstation it is important to ensure that (1) nothing is placed between the air-filter face and the object, material or product that is being handled and that needs to be protected and (2) you always work down-stream from the air-filter face, and do not let your hands or arms come between the item that is being protected and the air-filter face.

31.7 CONTROL OF THE STERILISATION PROCESS

31.7.1 General

Whatever type of sterilisation process is employed, it must be one that has been properly *validated*. Although it can and indeed has been argued that the intrinsically sound concept of validation has been pushed by some "experts" and regulatory authorities to extremes that boarder on the ludicrous,[12–14] it cannot reasonably be questioned that thorough validation of the process used for sterilisation is a crucial issue in the manufacture of sterile products.

Particularly rigorous attention should be given to validation when the adopted sterilisation method is non-standard or employs a non-standard cycle (That is, it is not one that is set out and described in the Pharmacopoeias). Where possible, heat sterilisation is the method of choice.

Before any sterilisation process is adopted, its suitability for the product and its efficacy in achieving the desired sterilising conditions in all parts of each type of load to be processed should be validated by physical measurements and by the use of biological indicators where appropriate. The process should be re-validated at scheduled intervals, at least annually, and whenever modifications have been made to, for example, process parameters or equipments.

It should be obvious, but in practice it has not always seemed so, that for effective sterilisation the whole of the material, or batch, must be subjected to the required treatment, and the process should be validated so as to demonstrate that this is achieved – each and every time. Thus, validated loading patterns should be established for all sterilisation processes that involve loading materials or products into a chamber or other form of steriliser. It is unreservedly insufficient merely to know that, say, in an autoclave, some part or parts of the load reach and hold the desired temperature for the specified time. The essential thing is that the coldest part of the coldest item in the coldest part of the load reaches the required temperature and holds it for the required time. If the achievement of those conditions entails other parts of the load reaching temperatures well in excess of those required for sterilisation, the design of the process and/or of the autoclave must seriously be called into question.

Biological and other indicators should be considered only as an additional method for monitoring a sterilisation process. They may be taken as an indication that a process may have failed, but because of their inherent variability they cannot, alone, be regarded as an indication that it has succeeded. If biological indicators are used, strict precautions should be taken to avoid transferring microbial contamination from them.

There should be a clear means of differentiating products that have not been sterilised from those which have. Each basket, tray or other carrier of products or components should be clearly labelled with the material name, its batch number and an indication of whether or not it has been sterilised. Indicators such as autoclave tape may be used, where appropriate, to indicate whether or not a batch (or sub-batch) has passed through a sterilisation process. Again, they cannot be taken to give a reliable indication that lot is, in fact, sterile.

The "raw" sterilisation records (*e.g.* time/temperature charts) of each sterilisation run should be retained as part of the batch-manufacturing record. They should be reviewed, along with the other batch documentation, as part of the batch-release procedure.

31.7.2 Sterilisation by Heat

Each heat-sterilisation cycle should be recorded on a time/temperature chart on a suitably large scale or by other appropriate equipment with suitable accuracy and precision, for example a digital printout device. The position of the temperature probes used for controlling and/or recording should have been determined during the validation, and where applicable also checked against a second independent temperature probe located at the same position. Where control of the cycle is automatic, the heat-sensing *control* probe should be independent of the *recorder* probe. (If the same

probe was used for both purposes and it was defective, it could actuate an inadequate cycle, yet still signal an apparently satisfactory one.)

Chemical or biological indicators may also be used, but should not take the place of physical measurements.

Care needs to be taken to guard against re-contamination of a sterilised load during the cooling phase. Any cooling fluid in contact with the product should have been sterilised, unless it can be infallibly shown that any leaking container would not be released for distribution. Air admitted before the chamber doors are opened should be filtered, and water used for spray cooling should be water for injection quality.

31.7.3 Steam Sterilisation

It is important to ensure that the steam used is of a suitable quality (''clean steam'') and does not contain additives, or other substances, which could cause chemical contamination of the product, material or equipment being sterilised.

Both temperature and pressure should be used to monitor the process. Where automated control and monitoring systems are used for these applications, they should be validated to ensure that critical process requirements are met. System and cycle faults should be registered by the system and observed by the operator. The reading from an independent temperature indicator should be routinely checked against the chart recorder during the sterilisation period. When a vacuum phase is part of the cycle, frequent leak tests should form part of the routine maintenance programme for the chamber used.

Items to be sterilised, other than products in scaled containers, should be wrapped in a material that allows removal of air and penetration of steam but that prevents re-contamination after sterilisation.

31.7.4 Dry Heat

It is important that there should be adequate air circulation within the chamber and a positive air pressure must be maintained to prevent the entry of non-sterile air. Any air admitted should be passed through a HEPA filter. Where this process is also intended to remove pyrogens, challenge tests using endotoxins should be used as part of the validation.

31.7.5 Ethylene Oxide Sterilisation

In sterilisation, direct contact between the gas and the microbial cells is essential for effective sterilisation. Organisms occluded in crystals, or coated with other materials, such as dried protein, may well not be killed. The nature and quantity of any packaging material can also markedly affect the efficacy of the process. Before exposure to the gas, the materials should be brought into equilibrium with the required temperature and humidity. Throughout the cycle, records should be made of the cycle time, temperature, pressure, humidity, gas concentration and total amount of gas used. These records should form part of the batch record, and used in the final evaluation of the batch for release/reject. Ethylene oxide sterilisation is an instance where use of biological indicators should be considered mandatory, rather than merely a possible useful adjunct. The generally recommended organism is *Bacillus subtilis* var. *niger*, deposited on a suitable carrier. The positioning of these indicators should be selected following validation studies to determine those parts of the load that are most difficult to sterilise. The information derived from the use of these biological indicators should form part of the batch-manufacturing record, as evaluated when making the final release/reject decision. After sterilisation, the load must be held, in a manner, which will prevent recontamination under ventilated conditions to allow ''degassing'' of residual gas and reaction products.

31.7.6 Radiation Sterilisation

During gamma irradiation sterilisation, the dose received should be monitored throughout the process by the use of plastic dosimeters inserted in the load in sufficient numbers, in packs sufficiently close together so as to ensure that in a continuous process there are always at least two dosimeters in the load exposed to the source. The standard red perspex dosimeters, as for example prepared by the UK Atomic Energy Authority at Harwell, give a reproducible, quantitative, dose-related change in absorbance, which should be read as soon as possible after exposure to the radiation. Electron beam sterilisation is rather more difficult to control. The dosimeters used are usually in the form of PVC films. In both cases, the dosimetry results should form part of the batch record. Biological indictors can be used, but *not* as a proof of sterilisation. Radiation-sensitive adhesive coloured discs are used, but only (repeat, *only*) as a means of indicating that a package has been exposed to radiation and not as a proof of sterilisation.

31.7.7 Filtration Sterilisation

In filtration sterilisation, which should only be used when it not possible or practicable to sterilise by other more secure means, non-fibre shedding filters, which are demonstrably capable of removing microorganisms, without removing ingredients from the solution or releasing substances into it, must be used. It is often advisable to use a (possibly coarser grade) pre-filter to first remove larger particles and thus reduce the load on the sterilising filter. Because of the potential additional risk of filtration as compared with other sterilisation methods, it is considered by many to be a sound practice to follow the first sterilisation grade filter with a second, in series, down-stream. The integrity of the sterilising (and sterilised) filter assembly, *in situ* (not just the filter in isolation), should be confirmed before use, and re-checked after use by such methods as the so-called bubble-point, pressure-hold or forward-flow tests. The time during which a sterile-filtered bulk solution is held, pending filling and sealing in its final container should be kept to a defined minimum, appropriate to the conditions under which the bulk-filtered solution is stored. Any one filter should not normally be used for more than one working day, unless a longer period of use can be justified by sound validation studies.

31.7.8 After Sterilisation

Of major importance is the need to avoid re-contamination of a sterilised product or material, and the mixup of sterilised with non-sterilised items. Ethylene oxide sterilisation is a special case where it is necessary to hold sterilised material under controlled ventilated conditions to allow any residual ethylene oxide and its reaction products to diffuse away. This presents additional problems in the prevention of re-contamination and mixup.

Besides the chemical analytical testing to confirm compliance with specification, sterile products also require to be subjected to further testing that is specific to this type of product. This includes

- Examination for particles
- Sterility testing
- Leak-detection testing, and possibly
- Pyrogen (or endotoxin) testing.

31.7.9 Examination for Particulate Contamination

The EC GMP Guide's revised (1996) Annex 1 – "Manufacture of Sterile Medicinal Products" requires that "Filled containers of parenteral products should be inspected individually for extraneous contamination or other defects", and adds that when this is a visual inspection

"it should be done under suitable and controlled conditions of illumination and background". It adds that operators engaged in this work should pass regular eyesight tests, with spectacles if normally worn, and be given frequent breaks from inspection. Pharmacopoeias (*e.g.* British, European and United States) have also variously set down requirements for the examination of filled parenterals for visible and sub-visible particles.

Visual inspection is a fallible process, relying as it must on subjective, hardly quantifiable judgments under conditions, which are difficult to standardise. Not only is it of doubtful value, it is also a dreary, time-consuming job that most workers would wish to avoid. It is not surprising, therefore, that various automated electronic methods have been developed. For a comparative review of the techniques and equipment available see Akers (1985).[15]

31.7.10 Sterility Testing

The severe statistical limitations of the compendial sterility test are generally acknowledged. There are also microbiological limitations, in particular the fact that there is no "universal" growth medium upon or in which all forms of microorganisms may be expected to grow. As generally practiced, sterility tests will not detect viruses, protozoa, exacting parasitic bacteria or many thermophillic and psychrophilic bacteria. Furthermore, organisms, which have been damaged but not killed by exposure to sub-lethal levels of "sterilisation", may not show up in the standard sterility test as they may require conditions for growth in terms of nutrients, temperature and time that the test does not provide.

Despite these acknowledged limitations, the test continues to be performed even by those who would accept that it has little real significance in terms of the quality of the product. This would appear to be due largely to regulatory requirements, and to a nervous perception of potential legal implications. The GMP guidelines appear to accept the limitations by declaring, for example, that "The sterility test applied to the finished product should only be regarded as the last in a series of control measures by which sterility is assured". The EC "Sterile Annex" (rev. 1996) considers that samples taken for sterility testing should be "representative of the whole batch, but should in particular include samples taken from parts of the batch considered to be most at risk of contamination". Examples given are of (a) samples taken from the beginning and the end of an aseptic run and "after any significant intervention" and (b) samples from the "potentially coolest part of the load" in a heat sterilisation.

There will be those who would consider that it would be difficult to encompass these requirements, within the limitations of the twenty unit sample that is usually taken, and they would be right. Akers[15] has considered alternative statistical sampling methods.

31.7.11 Leaks and Leak Testing

The Parenteral Society's Technical Monograph No. 3, "The prevention and detection of leaks in ampoules, vials and other parenteral containers"[16] rightly lays stress on the primary importance of *preventing* the formation of leaks.

The two main causes of leaks in ampoules are cracks in the glass and faulty sealing. Mechanical cracks can be caused by collision or abrasion of ampoules, one with another, or with or against other objects, during or after filling. In addition, thermal cracks can be caused in the glass through rapid cooling from higher temperatures, for example by contact of hot glass with cold machine parts. Such thermal cracks may develop immediately, or regions of stress may be induced, which develop into cracks later. Crack-inducing stresses can be caused during the original ampoule-forming operation, or during sterile product manufacturer, during heat sealing, ceramic printing and heat sterilisation. Faulty ampoule seals can arise from maladjustment or faulty setting of ampoule filling and sealing machines.

Methods aiding prevention are obvious: at all stages from the original forming of the ampoules to the dispatch of the finished product, careful steps should be taken to prevent impact and attrition of glass against glass, or with or against any other objects. Empty and filled ampoules awaiting further processing should be assembled neatly on their bases, and not just loaded haphazardly in basket loads. To prevent thermal stress cracks, contact must be avoided between hot glass and cold metal. Careful attention is necessary to machine adjustment, including flame settings, to avoid faulty sealing. With proper setting, draw sealing is less likely to give rise to faulty seals than tip sealing.

There are a number of ''traditional'' methods for leak-testing ampoules. They include various pressure/vacuum tests such as the common dye intrusion (or ''Dye Bath'') test, liquid loss and ''Blotting paper'' tests. These, and other techniques have been (and are) used, and they all have their limitations, even hazards – for example is that of dye solution entering an ampoule through a leak and then escaping subsequent detection.

Although not entirely free of problems and limitations, automated high voltage detection methods are more sensitive, and are not subject to the limitation of ''traditional'' method such as fallibility of human inspectors and hazards of undetected dye intrusion. They also have the further advantage that they can detect points of weakness, such as areas of thin glass, which at the time of testing are potential, if not actual, leaks.

With glass vials, the major stress should, again be upon prevention and not merely detection of leaks. Measures to prevent mechanical and thermal stresses and cracks are the same as that for ampoules. To minimise leaks arising from dimensional, physical and chemical inadequacies or incompatibilities, it is crucial that detailed and comprehensive specifications are agreed with suppliers of both vials and closures, and that compliance with specification is checked on all incoming deliveries.

In contrast with a fairly general acceptance of the need for 100% leak testing of glass ampoule products, a brief survey carried out in 1991/1992 indicated that 100% leak testing of glass vial products was the exception, rather than the rule. This is clearly unsatisfactory from a patient safety point of view, unless it can be shown that there is little if any possibility of leaks in filled and sealed glass vials, which does not appear to be the case. Pressure/vacuum tests can be applied to glass vial products, with the same limitations and problems for ampoules. However, based on the experience of the relatively few manufacturers of glass vial products that have tried the technique, it seems that automated high voltage detection is applicable to glass vial products. Such trials that have been conducted have shown that leaks do occur in production batches of filled glass vials, both in the vial body and in the closure system.

Leaks in LVP plastic containers can be caused by

- faults in the welding or sealing of the container when it is fabricated from the plastic sheets,
- inadequate ''fit'', or sealing, of components (tubes, closures, ports) attached to the bag,
- mechanical damage caused by contact with sharp or abrasive surfaces during filling, sterilisation and subsequent handling and
- pinholes or splits occurring during bag printing.

Pre-filled syringes and cartridges would clearly seem to represent a serious patient hazard if they have leaks. Somewhat disturbingly, however, it does appear that there is virtually no information available on the incidence and causes of leaks, nor on suitable methods of leak detection.

31.7.12 Pyrogen, or Endotoxin, Testing

The reaction in humans to injection of pyrogens can include chill, shivering, vasoconstriction, dilation of pupils, respiratory depression, hypertension, nausea and pains in joints and head, in addition to (or as a result of) the ''fire'', or rapid increase in body temperature, which the term

suggests. It is reasonable to assume that a patient receiving an injection is, in most cases, already ill. This additional stress to the system cannot be considered as anything less than highly undesirable.

Some substances, including some active drug substances (or "APIs", *e.g.* some steroids) and some viruses are pyrogenic *per se*, but in terms of sterile products manufacturing on an industrial scale, the most significant pyrogen is the bacterial endotoxin that is derived from the outer cell wall of certain Gram-negative bacteria. This substance is a complex, high molecular weight lipopolysaccharide, soluble in water and relatively heat stable. It can withstand autoclaving, and can pass through the 0.2 μm pores in the filters commonly used for sterilisation by filtration. Destruction, or removal, of microorganisms will not necessarily destroy pyrogenic endotoxin. There is thus another very good reason for keeping bacterial contamination at the lowest possible level at all stages in the manufacturing process, in addition to ensuring the lowest possible challenge to the sterilisation procedure. It is to reduce the chance of the presence of endotoxins. Prevention is, as ever, far better than later detection.

Pyrogenic contamination can arise at any stage in the manufacturing process. It may be present in starting materials, most notably in the water used to make solutions – hence the importance of good-quality water, produced by well-designed and monitored systems. It can be present on the surfaces of containers. It is unlikely to be present on glass containers, as manufactured, in view of the temperatures at which glass in blown or moulded, but it can be introduced by washing and rinsing glass containers with water that is not pyrogen free. It can be removed from glass containers by exposure to temperatures of 250°C or above, in for example a sterilising and de-pyrogenating tunnel. Once present in a solution, it is difficult if not impossible to remove. The answer is to not let it develop in the first place.

The traditional test for the detection of pyrogenic substances relies on the fact that the febrile response of rabbits resembles that of humans. The solution under test is injected into rabbits, and the rise, if any, in their rectal temperatures measured over the period of the test. The rabbit test has a number of disadvantages: it is a limit, rather than a quantitative test; it is time-consuming and subject to the variability and vagaries inherent in all biological test methods; and it cannot be used for solutions of substances that themselves prompt or inhibit a pyrogenic response.

A method that overcomes these problems, which can be used for quantitative determinations, and is more sensitive at low endotoxin levels is based on a discovery that a lysate of the amoebocytes from the blood of the so-called horseshoe crab (*Limulus polyphemus*, found mainly along the north-eastern seaboard of the American continent), in contact with bacterial endotoxin shows turbidity, or undergoes clotting (gelation). This is the *Limulus* amoebocyte lysate, or LAL, test. The LAL test kits are widely available from commercial suppliers. Although, at it is most simple, the turbidity/gelling end-point is determined visually and the method has been refined to permit more precise turbidimetric, colorimetric and nephelometric determinations (see Akers[15]).

31.7.13 Parametric Release

This concept, and its related terminology, emerged in the early- to mid-1980s and was originally related solely to the sterility (or otherwise) of terminally heat-sterilised products. That is, it did not originally bear upon other release criteria, or on the release of any other products, sterile or otherwise.

One of the first (if not *the* first) "official" publications on this subject is an FDA "Compliance Policy Guide" on "Parametric Release – Terminally Sterilised Drug Products". This guide provides a definition as follows:

> "*Parametric Release is defined as a sterility release procedure based upon effective control, monitoring and documentation of a validated sterilisation process cycle,* ***in lieu of release based upon end-product sterility testing***".

(Present writers emphasis)

Assuming the linguistic transplant "in lieu" means the same in United States as it does in Britain (and indeed in France) – then if "sterility release" may be based on "effective control . . ." *etc*., in place of a sterility test result, then the inverse corollary is surely implied that if a sterility test *has* been passed, then "effective control, monitoring and documentation of validated sterilisation process" is not necessary. This is contrary to all the principles of quality assurance in the manufacture of sterile products, and is thus presumably not what the FDA really meant.

This FDA guideline then goes on to list the actions that must be taken (and documented) as pre-conditions for parametric release.

In brief, they are given as:

(1) Validation of the cycle to achieve a reduction of the known microbial bioburden to 10^0 (sic), with a minimum safety factor of an additional six logarithm reduction. (Validation studies to include heat distribution, heat penetration, bioburden, and cycle lethality studies.)

(2) Validation of integrity of container/closure.

(3) Pre-sterilisation bioburden testing on each lot, pre-sterilisation, and checking comparative resistance of any spore-formers found.

(4) Inclusion of chemical or biological indicators in each truckload.

Worthy though the intention undoubtedly is, it is difficult to refrain from asking the question: Is not this (with the possible exception of the inclusion of biological indicators in *every* load) a list of things that should be done anyway, whether the lot is to be sterility tested or not?

This statement on "parametric release" provides an indication of the type and range of process parameters, which need to be considered before a product may reasonably be released without testing the end product for a specific quality characteristic. In this particular instance, a notably unreliable test procedure (the sterility test) may be abandoned, with at least a theoretical possibility of regulatory approval, in favour of a rigorous concentration of effort on actions that will provide a significantly higher level of assurance of sterility – an excellent notion in this context, and one that has been adopted (with official approval) by a few sterile products manufacturers. But they *are* surprisingly few. The reason for this probably lies in a not unfounded fear that, should action be taken for damages in the case of an alleged sterility failure, learned judges will probably consider "passed pharmacopoeial sterility test" – a better defence than technical and statistical arguments that they will not understand.

Recently, the European Commission has issued a new "Annex 17 to the EU Guide to Good Manufacturing Practice"[17], with a "proposed date for coming into operation" in January 2002.

Regrettably, as is so often the case with documents from this source, there is the usual poverty of linguistic expression and a liberal sprinkling of odd, ambiguous and paradoxical utterances. However, the oddest thing about this annex is its overall ambivalence. The standard form for documents of this type is that they are promulgated by regulatory bodies and directed at *manufacturers* as guidance on how to comply with GMP, both in general and in specific instances. This so-called Annex 17 reads rather more like a set of guidance notes for regulators considering whether or not to approve an application from a manufacturer to be permitted to release parametrically. As such, it would appear to have got lost and wandered into "Rules and Guidance for Pharmaceutical *Manufacturers* . . ." by mistake.

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